

## **TES02 - General Microscopy Techniques**

### **Scope**

This document describes general guidelines and general microscopic techniques used in the Trace Evidence Unit. The techniques outlined in this procedure may or may not apply to every microscope. For instructions on a specific microscope, please refer to the user manual for that microscope.

### **Safety Precautions**

No potential hazards are associated with these techniques.

### **Materials Required**

- Centering wrenches to fit microscope of interest
- Chemical resistant gloves
- Dust covers for microscopes
- Inert Dusting Gas
- Laboratory coat
- Lens cleaner
- Lens paper
- Polarized light microscope, with minimum 4x objective, 10x eyepieces, rotating stage, lower condensing lens
- Prepared slide of particulate material immersed in mounting media with cover slip
- Sable brush
- Stereobinocular microscope, with minimum magnification of 4 diameters
- Water
- White polystyrene foam (small grain type)
- Additional materials may be used at the discretion of the examiner

### **Standards and Controls**

Not applicable.

### **Procedure**

#### **1. General Care and Maintenance of Microscopes**

The optical components of the microscopes should be kept meticulously clean.

**1.1** Replace the dust cover on the microscope at the end of each day.

**1.2** Clean the external surfaces of objectives, eyepieces and condensers.

- 1.2.1** Gently blow dust away, using a can of Inert Dusting Gas, or using an absolutely clean soft sable brush.
- 1.2.2** After blowing away dust, use lens paper, dampened with water or lens cleaner to remove fingerprints, grease, oil and dirt. Never use alcohol on lenses.
- 1.3** White polystyrene foam (small-grain type) is recommended by some microscope companies for removing residues of immersion oil, skin grease and solvents. Break off a small piece and press a projecting part of it against the dry lens, rotating it as co-axially as possible with the objective. Any adhering foam granules can then be removed by blowing them away or using an absolutely clean sable brush.
- 1.4** Special care is required when working with acids and other chemical reagents. Their contact with the objectives should be strictly avoided. Clean the objectives (and other contaminated areas) at once after any accident. Even when particles are under a cover glass, there is a continuous stream of vapor from the corrosive substances that will impair the optical quality of the objective lens. Do not subject the lenses to prolonged exposure to these vapors.

## **2. Microscope Alignment**

### **2.1 Modified Köhler illumination is used with polarized light microscopes.**

- 2.1.1** If possible, align the light source so that the field of view is evenly illuminated while viewing through the microscope without a specimen on the stage.
- 2.1.2** Fully open the field and aperture diaphragms, if present.
- 2.1.3** Adjust the interpupillary distance (IPD).
- 2.1.4** Place specimen on the stage.
- 2.1.5** Using only one eyepiece focus on the preparation using fine and coarse adjustment knobs on microscope. Adjust the other eyepiece so that it is parfocal with the first.

### **2.2 For a Microscope Containing an Eyepiece Reticle**

- 2.2.1** Without looking into the eyepieces, turn the eyelenses fully counterclockwise until they stop.
- 2.2.2** While looking into the eyepiece containing the reticle, adjust the eyepiece containing the reticle until the reticle comes into focus.

- 2.2.3** Do not adjust the eyepiece containing the reticle again.
- 2.2.4** Using the eyepiece containing the reticle, focus on the test object.
- 2.2.5** Place a flat object beneath the objective.
- 2.2.6** Using the lowest magnification and the eyepiece containing the reticle, bring the object into focus.
- 2.2.7** Using the highest magnification, bring the image into precise focus.
- 2.2.8** Adjust the eyepiece which does not contain the reticle.
- 2.2.9** Using the lowest magnification and the eyepiece which does not contain the reticle, focus the eyelens.
- 2.2.10** Using the highest magnification, look at the object with both eyepieces, and bring the image into precise focus.

### **2.3 For a Microscope that Does Not Contain an Eyepiece Reticle**

- 2.3.1** Adjust the right eyepiece until it is in the center of its focusing range. Do not adjust the right eyepiece again.
- 2.3.2** Focus on the test object.
  - 2.3.2.1** Place a flat object beneath the objective.
  - 2.3.2.2** Using the lowest magnification and the right eyepiece bring the object into focus.
  - 2.3.2.3** Using the highest magnification, bring the image into precise focus.
- 2.3.3** Adjust the left eyepiece
  - 2.3.3.1** Without looking into the eyepiece, turn the eyelens fully counterclockwise.
  - 2.3.3.2** Using the lowest magnification and the left eyepiece, focus the eyelens.
  - 2.3.3.3** Using the highest magnification, look at the object with both eyepieces, and bring the image into precise focus, if necessary.

### **2.4 For a Microscope that Includes a Substage Assembly**

**2.4.1** Close field diaphragm by approximately one-half (1/2).

**2.4.2** Focus and center condenser.

**2.4.2.1** To focus the condenser, adjust the condenser focus knob until the image of the field diaphragm is in sharp focus.

**2.4.2.2** To center the condenser, adjust the condenser centering screws until the image of the field diaphragm is centered in the field of view.

**2.4.3** Open field diaphragm until just out of view.

**2.4.4** Remove eyepiece and open aperture diaphragm until just out of view. Replace eyepiece.

## **2.5 For a Microscope with Adjustable Objectives**

**2.5.1** Place a prepared slide containing small particles on the microscope stage.

**2.5.2** Using the lowest power objective, focus on a single particle.

**2.5.3** Move the slide so that the particle of interest is in the center of field of view.

**2.5.4** Rotate the stage. The particle should remain in the center of rotation. If the particle moves away from the center of the rotation:

**2.5.4.1** Rotate the stage until the particle is furthest from the center of the field of view.

**2.5.4.2** Adjust the objective centering wrenches until the particle is half way between its original position and the center of the field of view.

**2.5.4.3** Repeat steps 2.5.4.1 through 2.5.4.3 until the particle remains in the center of the field of view upon rotation of the stage.

**2.5.5** Repeat steps 2.5.2 through 2.5.4.3 for the remaining objectives.

## **3 Color Balancing – Comparison Microscopes**

**3.1** After performing modified Köhler illumination on both sides of the comparison microscope, place a known sample containing matching colored fibers on both sides of the comparison microscope.

**3.2** Adjust the light voltage (if applicable), aperture diaphragm, and field diaphragm until the background color is the same.

**3.3** If so equipped, adjust the Leitz Variolum Illumination Adapter until the colors appear the same in both sides of the comparison microscope.

### Limitations

Not applicable.

### Comments

Not applicable

### Documentation

The following worksheet(s) shall be generated and managed:

Casework Documentation
Not applicable.

### References

Nikon, How To Use A Microscope And Take A Photomicrograph, Nikon Corporation, 1998.

Patzelt, Walter J., Polarized Light Microscopy, 3rd edition, Ernst Leitz Wetzlar GmbH., 1985.

Möllring, F.K. Microscopy From The Very Beginning, Carl Zeiss, Oberkochen, West Germany.

Handbook of Incident Light Microscopy, Carl Zeiss, Oberkochen, West Germany.

Determann, H. and F. Lepusch, The Microscope and its Application, Ernst Leitz Wetzlar GmbH.

Delly, J.G., The Michel-Lévy Interference Color Chart-Microscopy's Color Key, *Microscope* 37, 89-102, 1989.

Delly, J.G., Photography through the Microscope, Eastman Kodak Company, 7<sup>th</sup> Edition, 1980.

DeForest, Peter R., Foundations of Forensic Microscopy. In: Forensic Science Handbook, 2<sup>nd</sup> edition, R. Saferstein (ed), Prentice-Hall, Inc. pp 215-319, 2002.